REMARKS

This is in response to the Office Action mailed December 10, 2009. Claims 1-22 are pending. Claims 18-20 have been withdrawn from consideration. Claims 11 and 14 are herewith canceled without prejudice or disclaimer. With entry of this amendment, claims 1-10, 12, 13, 15, 16, 17, 21 and 22 are active. No new matter is added with the amendment to claims 1, 2, 4, 7, 16, 21 and 22.

I. Claim rejections – 35 USC § 112

The Examiner rejects claims 2, 3 and 22 under 35 USC § 112, first paragraph, as failing to comply with the written description requirement. The Examiner has objected to the unit IU/L in claim 2. Applicants traverse this rejection. In the International application, the unit of measure for catalase was "u", which means unit. IU simply refers to International Unit and one of skill in the art would understand this nomenclature. Thus, Applicants respectfully request this objection be withdrawn. The Examiner also has objected to the term "about" in claims 2, 7, 16, and 22. Applicants respectfully traverse this rejection. In further response, applicants have deleted this term thereby rendering this objection moot.

The Examiner also objects to claim 7, stating that there is no antecedent basis for "in addition to an induction step for at least one surface antigen..." Applicants respectfully traverse this objection but believe this objection is rendered moot in view of the amendment to claim 7

The Examiner objects to claim 11, stating that the term "medium" does not have an antecedent basis. The Examiner also asserts that claim 11 does not further limit claim 1. In response, applicants have canceled claim 11, thereby rendering this objection moot. In view of the above amendment and comments, applicants respectfully request the Examiner to reconsider and withdraw all rejections under 35 USC § 112.

The Examiner objects to claim 14, arguing that "detection and analysis ..." lacks

an antecedent basis in claim 1. In response, applicant has canceled claim 14, thereby rendering this objection moot.

II. Claim rejections – 35 USC § 103

Claims 1, 4, 5, 6, 11, 14, 15 and 17 remain rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg" in view of Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle"). The Examiner also newly rejects claims 2-3 and 21-22 under 35 USC § 103(a) as being unpatentable over Berg in view of Pyle and also in view of Strenkoski (US 5,843,699) and Heck (US 3, 704,204) and Ray, Bibeck (*Injured Index and Pathogenic Bacteria* 1989 CRC Press Inc. Boca Raton, FL page 78) and Patel (*J. of Food Protection* 1995 58(3): 244-250). Applicants respectfully traverse these rejections.

The primary reference in the above rejections is Berg. The secondary references are cited for teaching various aspects of some of the steps of the independent claims or aspects of the dependent claims. Applicants have argued previously that the method of Berg is essentially different from that of the claimed invention and that the secondary references fail to cure the deficiencies in Berg. According to the Examiner, applicants' prior arguments with regard to Berg are not persuasive because the pending claims do not recite "strictly intracellular labeling of the microorganisms." The Examiner also notes that the claims recite "comprising" so as to not exclude labeling at other sites. Finally, the Examiner states in the office action at page 7, first paragraph, that Berg "gives the option of contacting the enzyme insider or outside of the microorganism with a fluorogenic substrate." Applicants disagree with this interpretation of the claims and with the Examiner's interpretation of Berg.

Claim 1 recites:

d) fluorescently labeling the microorganism by adding to the sample containing the microorganisms at least one substrate comprising one part specific to the enzymatic activity to be indicated and one fluorogenic label, wherein the

transformation of the substrate takes place inside the microorganism and wherein the fluorescent product resulting from the fluorogenic label is retained in the microorganism...

It is clear from part d) of claim 1 that the label occurs and is retained within the cell. The Examiner's conclusion to the contrary is based upon a misinterpretation of claim 1. In order to further clarify this feature of the invention, applicants have amended the preambles of claims 1 and 21 to introduce "intracellularly labeled" to describe the microorganisms detected and counted by the claimed method.

The intracellular nature of the labeling of microorganisms in accordance with the claimed method is relevant. The intracellular labeling permits the counting of the fluorescently labeled microorganisms by a technique selected from the group consisting of flow cytometry, filtration cytometry, and fluorescene microscopy. These techniques allow the detection of the fluorescence of each fluorescent cell. Accordingly, the background fluorescence is not registered, thus improving sensitivity and specificity.

But, as noted above, the Examiner believes that Berg "gives the option of contacting the enzyme inside or outside of the microorganism with a fluorogenic substrate." First, applicants point out that the present claims, as explained above, are directed to strictly intracellular labeling. Berg does not disclose or suggest strictly intracellular labeling and the association of such labeling with numeration or counting of microorganisms. Additionally, Berg does not disclose the retention of the label within the cell; whereas, the current claims require the label to be retained within the cell. The fact that the Berg may suggest that the substrate contact the enzyme inside or outside the microorganism is not the same thing as retaining the label within the cell. Rather, Berg discloses the release of the fluorescent molecule and the measurement of the velocity of the emitted fluorescence (page 7, first paragraph). The release is achieved with permeability agents (see page 6, lines 12 to 15), in particular with sodium lauryl agent (see page 7, lines 13 to 18). These permeability agents can cause lysis of the cells. These steps in Berg's method teach away from what applicants claim, i.e, the retention of

the intracellular label.

Applicants also point out that the method disclosed in Berg necessitates a series of measurements and their comparison with a standard curve to determine the concentration of microorganisms in the sample (see claim 1, step f)). Moreover, the released fluorescence in the medium creates a background fluorescence which does not permit a specific numeration or counting of the microorganism.

In sum, the method of claim 1 relates to **counting each microorganism** whereas Berg's method relates to the **quantification** of a fluorescent signal. Consequently, Berg does not disclose or suggest the claimed method.

The Examiner's reliance upon Pyle and the other secondary references does not cure Berg's failure to suggest the claimed invention. Applicants rely upon their previous characterization of Pyle. Namely, Pyle discloses a method involving immunomagnetic separation and fluorescence quantification after immunofluorescent labeling (page 37, line 32 to page 38, line 23). However, Pyle does not disclose or suggest a step of selectively enriching the microorganism sought in the sample, nor does it disclose or suggest the use of a substrate comprising one part specific to the enzymatic activity, nor does it disclose or suggest the induction/activation of at least one enzymatic activity of the microorganism. Finally, nowhere does Pyle disclose or suggest the strictly intracellular labeling that is required for the numeration and counting microorganisms, according to the claimed invention. Likewise, the other cited art, either alone or in combination with Berg and Pyle, fail to teach or suggest these claimed features of the invention.

In view of the above explanations, applicants respectfully request the Examiner to reconsider and withdraw all of the rejections for obviousness.

CONCLUSION

Should the Examiner believe that anything further is necessary in order to place this application in better condition for allowance, the Examiner is requested to contact the

undersigned at the telephone number listed below.

In the event that an extension of time is necessary to prevent abandonment of this application, then such extension of time is hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account No. 01-2300 referencing docket number 029440.00009.

Respectfully submitted,

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